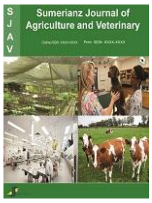
Sumerianz Journal of Agriculture and Veterinary, 2019, Vol. 2, No. 7, pp. 40-48
ISSN(e): 2617-3077, ISSN(p): 2617-3131
Website: https://www.sumerianz.com



Original Article Open Access

Menu Script Field: Response of Soybean to Water Deficit and Spider Mites During Seed Filling Stage

Natalia Georgieva*
Professor, Department of Technology and Ecology of Forage Crops, Institute of Forage Crops, Pleven-5800, Bulgaria

Ivelina Nikolova
Professor, Department of Technology and Ecology of Forage Crops, Institute of Forage Crops, Pleven-5800, Bulgaria

Abstract

Morphological, physiological and biochemical responses of soybean were investigated under influence of water deficit and spider mites, imposed during seed filling stage. The pot experiment was conducted under greenhouse conditions and included 3 factors: factor A - mode of irrigation (water deficit and irrigation), factor B - mites (presence or absence of mites) and factor C - imidacloprid (with and without imidacloprid treatment). Imidacloprid was used due to its protective effect in conditions of abiotic and biotic stress. The results showed that the interaction of the stress factors led to a reduction in the leaf area (46.7%), relative water content (17.9%), total content of photosynthetic pigments (39.8%) and to an increase in density of leaf trichomes (23.1%) and content of water-soluble sugars (128.6%). Soybean aboveground biomass was characterized by reduced protein content (by 17.6%) and in vitro digestibility of dry matter (7.2%) and slightly increased synthesis of NDF, ADF and cellulose. Negative consequences of the mites in conditions of both modes of irrigation (+ WD, -WD) showed that they are more pronounced under water deficit than in irrigation regarding plant leaf area, total pigments and biomass protein conten. Treatment with imidacloprid had a positive effect on the stressed plants (+ WD + M + I). It favored the synthesis of water-soluble sugars, leaf pigments and basic parameters such as CP, CF and NDF in the soybean biomass as well as increased the size of leaf area. In future, more investigations are needed to study the effect and the possibility to use different substances to minimize the consequences of biotic and abiotic stress.
Keywords: Soybean; Water Deficit; Mites; Trichomes; Leaf Area; Nutritive Value.

1. Introduction

Water stress, more than any other environmental factor, strongly influences plant growth and development, and decreases productivity and performances of the crops [1]. According to Cia, *et al.* [2], it is the major yield-limiting factor of crop plants and has the ability to decrease 50% of yield in the most major crops [3]. Specific responses of the plant to water deficit are determined by the amount and ratio of water loss, duration of water stress and the stage of development of crop [4].

Soybean (Glycine max (L.) Merrill) is a globally important commercial crop, grown mainly for its protein, oil and nutraceutical contents. The seeds of this legume contain 40% protein and 20% oil. Each year soybean provides more protein and vegetable oil than any other cultivated crop in the world [5]. It is important and traditionally grown in Bulgaria. Racz [6], determined it as a moisture-demanding crop for which is established a significant decrease in yield during periods with unbalanced rainfall and a lack of moisture. Soybean is particularly sensitive to the moisture lack during the blooming process (growth stages R1 and R2) and during the legume and seed growing process (growth stages R3 – R6) [7]. Mederski, *et al.* [8], claims that water stress during the blooming process (growth stages R1 and R2) and legume growing process (growth stages R3 and R4) is a factor responsible for a flower and legume abortion while the stress during seed growing process (growth stages R5 and R6) leads to a reduction in the seed size.

Spider mites are serious pests of soybean and cause a signicant yield loss. Hot and dry weather favors reproduction and survival of this pest because this type of weather is unfavorable for pathogenic fungi (Neozygites), a primary biological control agent that keeps spider mite populations at subeconomic levels [9]. Under stress (biotic or abiotic) conditions, the plants present a series of changes in their morphology, physiology and biochemistry, negatively affecting their growth and productivity [10-14]. Only a few of the studies considered the interactions between one of the main pests – spider mites and crops' water regime [15-18]. The knowledge about the influence of water deficit on important characteristics of the nutritive value of legumes are also limited [19].

Studies of some researchers in recent years were encouraging and showed that some compounds – jasmonic acid [20], methyl jasmonate [21, 22] – can improve the growth and manifestations of plants under abiotic stress. In conditions of a field trial [23], established that another chemical compound (imidacloprid) had protective action under abiotic and biotic stress. After imidacloprid application in terms of drought stress, plants (barley, pepper) improved their growth and productivity.

With this experiment, we aimed to investigate the response of soybeans to water deficit and spider mites attack, as well as the possibility of reducing the negative effects of the stress through treatment with imidacloprid.

*Corresponding Author

2. Materials and Methods

The pot experiment was conducted under greenhouse conditions in the Institute of Forage Crops during the period 2011-2012. The pots used were type Wagner as in each one 4 soybean plants (variety Richy) were grown. Various manifestations (morphological, physiological and biochemical) were observed under the impact of the following factors: factor A - mode of irrigation (water deficit and irrigation), factor B - mites (presence or absence of mites) and factor C - imidacloprid (with and without treatment with imidacloprid). All plants received an equal amount of water to maintain optimum soil moisture by the end of phase R5 [24]. After this phase, a 10-day water deficit was imposed by reducing 1/2 of irrigation rate. The optimum soil moisture was restored after the 10-day drought, as some of the variants were treated with imidacloprid (150 g/ha).

Spider mites (Tetranychus atlanticus Mc Gregor) on soybeans appeared at stage R1 (1.0 mobile forms/leaflet) and their population density remained low until the end of stage R4. During the stage R5, the mite density increased, reaching to 69 mobile forms. From the imposition of water stress (the end of stage R5) to its termination, population density in variants with mite attack was respectively 124 mobile forms/leaflet in conditions of water deficit and 65 mobile forms/leaflet in irrigation conditions.

The experimental variants were 8, as each variant was in 11 replications:

+ WD + M + I: water deficit, mites, imidacloprid treatment
+ WD – M + I: water deficit, without mites, imidacloprid treatment
+ WD + M – I: water deficit, mites, without imidacloprid treatment
+ WD – M – I: water deficit, without mites, without imidacloprid treatment
- WD + M + I: irrigation regime, mites, imidacloprid treatment
- WD – M + I: irrigation regime, without mites, imidacloprid treatment
- WD + M – I: irrigation regime, mites, without imidacloprid treatment
- WD – M – I: irrigation regime, without mites, without imidacloprid treatment

In the variants without mite infestation was used an acaricide.The calculated coefficient of stress intensity (D), according to the formula of Fischer and Maurer [25] based on the imposed water deficit and the mite attack in the present experiment had a mean value (D = 0.55).

Relative water content method of Barrs and Weatherley [26], and content of water soluble sugars [27] in the leaves of soybean plants were determined 7 days after the restoration of the irrigation regime and imidacloprid treatment, and content of plastid pigments (method of Zelenskiy and Mogileva [28], – 10 days after the recovery of irrigation regime and treatment with imidacloprid. These determinations were measured on the third mainstem leaf below apex. The size of leaf area (cm2 plant-1) (the method of Sinyakova and Ivanova [29], and the leaf trichome density (number cm-2) were recorded at the end of growth stage R6. To determine the leaf trichome density in the eight variants were used the latest fully developed leaves in the mainstem of soybeans, randomly selected from 11 plants. Measurements were made with binocular Amplival, on the upper and lower surfaces of the leaves, and the data were averaged.

The principal chemical composition of the aboveground biomass of soybean plants was determined at the end of stage R6. The general composition was determined as crude protein (CP) by Kjeldal method and crude fiber (CF) by Weende system [30]. The plant cell walls components content was determined as Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL). Cellulose as cell wall component, contained in fiber fraction, was presented as follows: Cellulose = ADF - ADL. The degree of lignification was presented as relation of ADL and NDF / 100. Enzymatic in vitro digestibility of dry matter (IVDMD, %) was performed by two stage pepsin-cellulase method of Aufrere [31].

Statistical data processing was conducted by the Software Statgraphics Plus.

3. Results

The main factors which were the subject of this study, mode of irrigation and mites, affected some morphological (leaf trichomes, leaf area) and physiological parameters (relative water content, content of water soluble sugars) of soybean plants (Table 1).The trichomes density (number cm-2) of the soybean leaf surface was averagely 373 and their number in conditions of water deficit (+ WD - M - I) was 30.9% statistically significant greater compared to the same under irrigation (- WD - M - I). The influence of mites on this indicator was significantly weaker and was determined by the mode of irrigation: under water deficit the difference in density of leaf trichomes in terms of presence and absence of mites was more substantial, albeit non-significant (respectively 394 and 419 number cm-2), while in irrigation conditions this difference was negligible (317 and 320 number cm-2).

The soybean leaf area was considerably affected by water stress and mites. The reduction under the self-action of water stress (+ WD - M - I) and mites (- WD + M - I) was respectively 23.3 and 12.4% compared to the relevant control variation (- WD - M - I), while the interaction of both factors had a cumulative effect and led to a sharp decrease of 46.7%. On the other hand, the decrease in the leaf area size under the action of mites in water deficit conditions had a considerably higher value – 30.5% (variants + WD + M - I and + WD -M - I) than the reduction in irrigation – 12.4% (variants - WD + M - I and - WD - M - I). Imidacloprid treatment with had a positive effect on this indicator and to some extent compensated the adverse effects of the imposed two types of stress, increasing leaf area by 10.6%. In conditions of the absence of mites and under irrigation (- WD - M + I) the increase was 15.1% as compared to the corresponding variant (- WD - M - I).

The method of Barrs and Weatherley [26], for determining the RWC assesses the water content of the leaf tissue as compared with the maximum water content that can be maintained at full turgor. This indicator is a measure of water deficiency in the leaves.The lowest RWC was characteristic for the leaves which were imposed of water stress

× mites interaction (+ WD + M - I), whose value was slightly higher than the value at the wilting. The decrease compared to the corresponding variant under irrigation and without mites was 17.9% (- WD - M - I). Plant leaves in all variants under irrigation had a higher RWC (average 93.10%) than those under water deficit (84.45%), which determined their greater water keeping ability. Similarly, the plant leaves grown in the absence of mites, irrespective of the regime of irrigation, also maintained higher values of RWC (92.26%) in comparison with the mite-stressed plants (85.29%). The increase in RWC of soybeans after imidacloprid treatment in conditions of water deficit and mites (+ WD + M + I) was relatively low (6.4%) and statistically insignificant.

The content of water soluble sugars in the soybean leaves showed substantial variation between variants in the ranges of 0.70 (- WD - M - I; - WD - M + I) to 2.50 (+ WD + M + I). Very well expressed and significant was the impact of both factors, water deficit and mites under whose influence the soluble sugar content tended to increase, respectively, with 128.6 and 100.0%. Effect and increase after imidacloprid treatment in the content of soluble sugars in plants under the combined influence of water deficit and mites (+ WD + M + I) was established compared to the control variant (+ WD + M - I).

Population density of spider mites in variants +WD+M+I, +WD+M-I, -WD+M+I and

-WD+M-I, traced during 20 days after termination of the water deficit and imidacloprid treatment varied in relatively wide limits and had mean values of 20.14 to 45.90 mobile forms per 10 cm2 leaf area (Fig. 1). It was obvious a higher density of mites in conditions of water deficit than in irrigation (with 78.1%) and manifested acaricide action of imidacloprid. After using imidacloprid, number of mites decreased by 34.0 and 11.0%, respectively, in water stress and irrigation. The spider mites density was in negative correlation with the leaf area size (r = -0.888) and the relative water content (r = -0.983), and positively correlated with the leaf trichome density - (r = 0.820).

Soybean plants, subjected to the impact of water stress and mites, showed changes in the content of leaf photosynthetic pigments (Table 2). In comparing between the effect of both factors on the total pigment content it was found that the water stress (in absence of mites and imidakloprid treatment) decreased the total pigment content by 23.2% while the presence of mites (in absence of water stress and imidacloprid treatment) - by 19.0%. The effect of double stress (water deficit, mites without imidacloprid treatment) was almost twice as high and the decrease in the total content of pigments was 39.8%. It should be noted that the highest sensitivity to the impact of the two factors (water deficit and mites) manifested chlorophyll a, followed by chlorophyll b and carotenoids (a reduction of respectively 41.0, 39.3 and 35.8%). The negative consequences of the mites infestation under water deficit on the total pigments content were more pronounced than under irrigation conditions which is logical and understandable, given the high density of mites in these variants. A positive effect from the use of imidacloprid and an increase in the content of total pigments was observed only in plants which undergo water deficit while under irrigation such effect was not found.

The forage quality is an important feature of leguminous fodder crops. The chemical composition of aboveground biomass of soybeans (Table 3) during seed filling showed little variation in terms of the content of crude protein and crude fiber. Plants of all variants in water deficit conditions formed a biomass with a lower protein content (average of 153.08 g kg-1 DM) in comparison with the plants under irrigation conditions (average of 172.95 g kg-1 DM). The interaction water deficit and mites (+ WD + M - I) decreased the protein content to the greatest extent (by 17.6%). In conditions of water deficit, soybean plants exhibited much greater sensitivity to the presence of mites in comparison with the plants under irrigation, as in the first case the protein content was decreased by 15.5%, and in the second case was recorded a slight increase. Unlike the crude protein content, the changes in the content of carbohydrates under influence of studied stress factors were less pronounced but followed the same trend of decrease. The accumulation of crude fiber, NDF, ADF and cellulose was decreased respectively by 0.4, 4.7, 0.9 and 8.0%. The degree of lignification was negatively influenced by the regime of irrigation and mites as both factors contribute to it increasing. In vitro dry matter digestibility, as the main indicator of the quality of the feed from the standpoint of animal nutrition varied between 63.76 - 67.32% under water deficit and from 66.50 to 69.87% under irrigation, with decline under the influence the stress factors by 7.2%. The treatment with imidacloprid favored the synthesis of basic parameters such as CP, CF and NDF, increasing their content, respectively with 11.0, 10.5 and 4.2% for the plants in stress conditions (+ WD + M + I) as compared to plants from variant + WD + M - I, but did not affect the digestibility of soybean biomass.

4. Discussion

Although the plant growth under water stress is reduced overall, it is well-known that plants have a suite of morphological and physiological adaptations that allow them to survive under water stress. The degree of adaptation to drought may vary considerably between species [32]. In our conditions, the newly formed leaves of plants in all variants with imposed water deficit are distinguished with increased trichome density (averagely 421 number cm-2) as compared to plants grown under irrigation (averagely 325 number cm-2). The increase in leaf hair density observed in water stressed plants agrees with the results obtained in wheat by Quarrie and Jones [33], in Lotus creticus by Bañon, *et al.* [34], and in other species by Stancheva [35]. Some authors have suggested that the leaf hairs may improve leaf water status by entrapping and retaining surface water, thus, assisting in its final absorption into the mesophyll, or reducing water loss by increasing the resistance of the boundary layer [36]. In the opinion of other researchers [37] there is a negative correlation between the trichome density and environments with low water content. According to Hameed, *et al.* [38], the varietal identity is of the essence in this case.

Leaf area development is an important factor in crop production because it affects the amount of radiation intercepted and, therefore, plant growth [39]. According to Santos and Carlesso [40], the most prominent responses of plants to water deficits in terms of morphological processes are decreases in leaf area and acceleration of the senescence and abscission of leaves. The reduction in leaf area as a result of deficit irrigation has been considered an avoidance mechanism, which permits minimising water losses [32]. Many researchers have reported about strongly affected leaf area in soybean varieties due to water deficit by indicating different values. Catuchi, *et al.* [41], have observed a reduction of approximately 40% of leaf area per plant compared to control plants under water deficits induced at the V4 stage [42], – 79% reduction after deficit in irrigation regime at the V4 stage [43], – 60% decreased leaf area after water deficit induced 21 days after emergence. In addition [42], stated that the decrease in leaf area in water-stressed plants was due to both senescence and abscission of lower leaves and reduced leaf emergence and expansion. The consequences of water stress are determined mainly by the stage in which is induced, and its intensity. The water deficit in our study was introduced at a later stage so that the effect was expressed to a lesser extent. Regarding the second factor (mites) deserves to be discussed the different reduction of leaf area in both modes of irrigation. Due to the higher density of mites in water deficit, the reduction in these conditions was more pronounced. The reasons why mites prefer water-stressed plants are many (strengthened synthesis of nitrogen compounds and sugars, increased the temperature of leaf surface) and as a whole promote their nutrition, development, and reproduction [44-47]. The stimulatory effect of imidacloprid in relation to the leaf surface, observed in the present experiment was in confirmation of the studies of Thielert [23]. The author found a significant leaf area growth following imidacloprid application after short-term drought stress.

According to Jaleel, *et al.* [48], water stress is characterized by reductions in leaf water potential, RWC and loss of turgor, which decreases stomatal conductance and cellular expansion, consequently constraining plant growth. Severe water stress can result in impairment of photosynthesis, metabolic disorders and ultimately death of the plant. In addition, other factors, such as pathogen attacks, may also contribute to differences in the amount of water in plants by interfering with their development and reducing their productivity [49]. The measurement of RWC in leaves of soybean variety Richy, 7 days after recovery of the irrigation regime, showed lower values in comparison with those that were not subjected to water deficit. It is evident that despite recovering irrigation regime, they cannot reach values before stress introducing, which, according to some authors [50, 51] is due to some structural changes occurring in the plants, ensuring adaptation to drought. Our data are consistent with the results of Jie, *et al.* [52], who observed changes in the RWC in different varieties of Boehmeria Jacq. in conditions of 10-day water deficit. The authors found that 15 days following the cessation of water deficit and rewatering, the relative water content in all varieties was lower compared to unstressed control plants. The changes in RWC under the mite influence are mainly related to the process of feeding, in which the leaf epidermis and cuticle are hurt. The damaged leaves transpire more water compared to the intact leaves, reducing in this way their water potential [53]. Our results showed that the decrease in RWC as a result of the mite feeding was more strongly expressed under water deficit (10.6%) than under irrigation (6.9%). The same dependence was established by Sadras, *et al.* [18], in similar experiment with soybean in conditions of water deficit and infestation by Tetranychus urticae Koch. For diminished RWC in different crops due to water deficit or mite attacks reported [54]. Haile and Higley [9], and [55].

Carbohydrate mechanism is very sensitive to changes in plant water status [56]. Increased sugar levels in the plant leaves during drought conditions are established by many researchers [57-59]. According to Chaves, *et al.* [60], they are result of regulation of the synthesis and translocation of sucrose, contributing to osmotic adjustment and enabling the maintenance of turgor in meristematic regions. These responses lead to restoration of cellular homeostasis, which increasing plant survival under stress. As a result of mite feeding [10, 11, 53] observed an increase in plant water stress and accumulation of soluble leaf sugars which play a part in enhancing mite reproduction. Probably this sugar accumulation helps mite-injured leaves to maintain cell turgor pressure through an osmotic adjustment. Increased sugar levels after mite or insect attacks also have been reported by Hildebrand, *et al.* [61], Wheeler [62], Sailaja, *et al.* [63], and Tomczyk [64]. Higher sugar content as a result of imidacloprid treatment was found in both irrigation regimes (water deficit and irrigation) but only in the mite presence, as the difference between the treated and untreated variant was more pronounced under water deficit (increment by 7.1 and 56.3 % respectively under irrigation and water deficit). After foliar application of another plant growth regulator (methyl jasmonate) in soybeans under water deficit [22] observed accumulation of total soluble sugars (as compared to control plants), leading to improved resistance against water stress.

The total pigment content (chlorophyll a, chlorophyll b and carotenoids) was affected negatively by stress conditions in our experiment. Brevedan and Egli [65], indicated that the water stress accelerates strongly the senescence of leaves which was confirmed from the rapid decrease in the content of leaf pigments. The dependence was found in cotton [66], lupine [67], wheat [57] and other crops. The decrease in chlorophyll content under water stress is a commonly observed phenomenon [68]. It might be due to reduced synthesis of the main chlorophyll pigment complexes [69] or destruction of chiral macro-aggregates of light harvesting chlorophyll ‘a’ or ‘b’ pigment protein complexes which protect the photosynthetic apparatus [70] or due to oxidative damage of chloroplast lipids, pigments and proteins [71]. Spider mites also reduced the pigment content of soybean leaves as the reduction was influenced to some extent by the irrigation regime. They caused a greater reduction in water-stressed plants (21.6%) than in water-unstressed plants (18.9%). Several other studies have also demonstrated the adverse impacts of spider mite injuries to plants, which include reduced chlorophyll content [9, 11, 72]. The interaction water deficit × mites caused a significant reduction in the total pigment content as well as the individual components (chlorophyll a, chlorophyll b and carotenoids). The greater decrease in chlorophyll a content, observed during the study, was due to a higher sensitivity of this pigment to stress factors. In the least extent drought affected the content of carotenoids, which may be explained by their biological role to protect the chlorophyll from photooxidation under stress conditions [73]. The increase in the content of chlorophyll as well as RWC in soybean leaves after the restoration of irrigation regime and imidacloprid treatment are in accordance with the results of Han, *et al.* [74], observed in tobacco and pepper. The authors considered that imidacloprid protects plants, inducing in them tolerance to drought.

According to Emam, *et al.* [75], carbohydrates that represent ones of the main constituents of the dry matter are affected by drought stress terms. The NDF concentration gives an estimation of the structural part of the plant material (cellulose, hemicellulose and lignin) and is inversely related to the voluntary fodder intake. Acid detergent fibre include lignin and cellulose, and correlate negatively with cell wall digestibility [76].

Under conditions of drought stress [77], found reduced NDF and ADF concentrations in different forage legumes, but inconsistent changes in CP concentrations. The present results indicated that stress conditions decreased the content of CP, and to a lesser extent – the content of NDF, ADF and cellulose. The CP concentration in plant tissues was a result of N uptake and the development of biomass in time which was greatly determined by water availability [19]. Nakayama, *et al.* [58], found an impaired N uptake in soybean under drought. This explains why the CP concentration in the stress conditions was smaller than the corresponding concentrations in the control treatment. Küchenmeister, *et al.* [19], have established that drought effects on NDF (including cellulose, hemicellulose and lignin) were stronger than for ADF (including cellulose and lignin), which was confirmed in the present study. It might be explained by the fact that the hemicellulose concentration, as a part of NDF, was more affected by drought than cellulose and lignin [19]. Additionally, the ADF concentration in most legumes was approximately 100 g kg-1 lower than that of NDF [78] which was also established in our experiment (Table 3). A lower fibre concentration may lead to a higher herbage intake by the animals [78].

Mite stress also altered the nutritional status of the host plant and tended to decrease in leaf nitrogen content [79], NDF and ADF [80], total plant nitrogen and plant water content [81]. Generally, the decrease observed in main indicators of soybean chemical composition was accompanied by an increment of the lignification degree (by 6.9% under influence of WD and by 8.7% in mite presence) and a decrease of the in vitro digestibility (by 6.5 and 2.4% respectively). Such results were supported by Taiz and Zeiger [82]. The established in the course of the study enhanced synthesis of crude protein and leafy pigments in the soybean biomass after imidacloprid treatment was in line with our previous studies in winter vetch [83].

In terms of this experiment, the water deficit has a stronger influence on the morphological, physiological and some biochemical performances of soybeans, compared to spider mite infestation. The results of imidacloprid treatment were promising, but insufficient, and can probably be improved by its use in earlier stages of plant development and several time applications. In future, more investigations are needed to study the effect and the possibility to use different substances to minimize the consequences of biotic and abiotic stress.

5. Conclusions

Spider mites (Tetranychus atlanticus) and water deficit, imposed during seed filling stage, cause a number of morphological, physiological and biochemical responses in soybeans. Under the conditions of this experiment, the interaction of the stress factors led to a reduction in the leaf area (46.7%), relative water content (17.9%), total content of photosynthetic pigments (39.8%) and to an increase in density of leaf trichomes (23.1%) and content of water-soluble sugars (128.6%). Soybean aboveground biomass was characterized by reduced protein content (by 17.6%) and in vitro digestibility of dry matter (7.2%) and slightly increased synthesis of NDF, ADF and cellulose.

Negative consequences of the mites in conditions of both modes of irrigation (+ WD, -WD) showed that they are more pronounced under water deficit than in irrigation regarding plant leaf area, total pigments and biomass protein conten.

Treatment with imidacloprid had a positive effect on the stressed plants (+ WD + M + I). It favored the synthesis of water-soluble sugars, leaf pigments and basic parameters such as CP, CF and NDF in the soybean biomass as well as increased the size of leaf area.

Table-1. Leaf trichome density, leaf area, relative water content and water soluble sugars in soybean under conditions of water deficit and mite attack, 2011-2012

Variants	Leaf Trichomes			
Number, cm-2	Leaf area			
Cm2 plant-1	Relative			
Water content, %	Water			
Soluble sugars,%				
+WD+M+I	393 b*	531.94 a	83.64 ab	2.50 d
+WD-M+I	478 c	751.89 bc	87.64 bc	1.60 bc
+WD+M-I	394 b	480.87 a	78.59 a	1.60 bc
+WD-M-I	419 b	692.12 b	87.91 bc	160. c
-WD+M+I	329 a	872.91 cd	89.80 c	1.50 bc
-WD-M+I	332 a	1037.80 e	97.73 d	0.70 a
-WD+M-I	317 a	790.19 bcd	89.12 c	1.40 b
-WD-M-I	320 a	901.95 de	95.75 d	0.70 a
LSD0.015%	53.84	140.25	5.30	0.223

*Values within a column followed by the same letters are not significantly different

Figure-1. Density of spider mites in soybean under conditions of water stress and irrigation

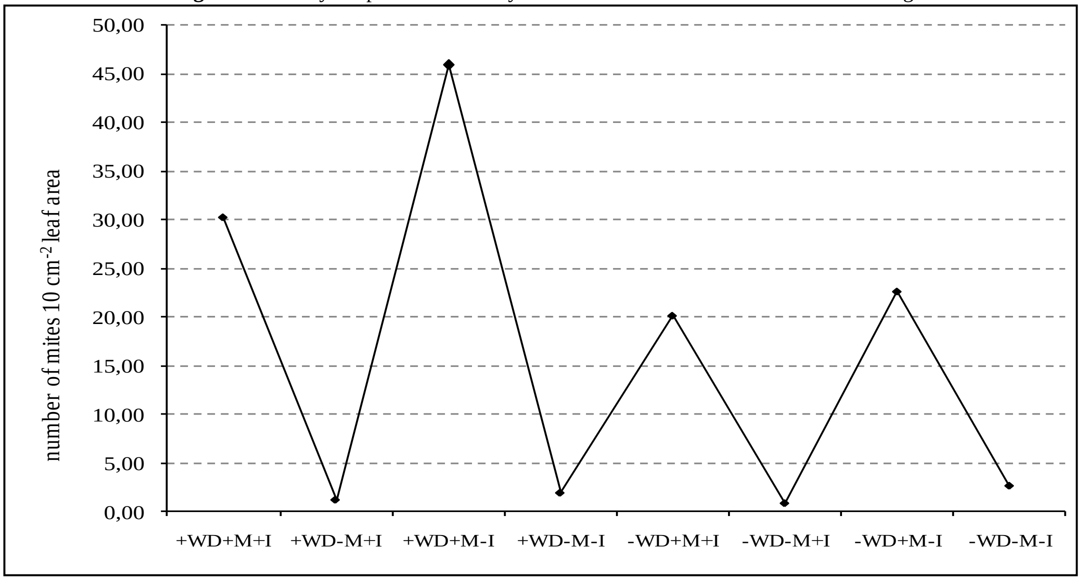


Table-2. Content of leaf pigments in soybean, mg/100 g fresh mass, 2011-2012

Variants	Chlorophyll a	Chlorophyll b	Carotenoids	total
+WD+M+I	7.200	2.808	1.714	11.721
+WD-M+I	8.214	3.243	2.062	13.518
+WD+M-I	5.732	2.431	1.366	9.529
+WD-M-I	6.840	3.433	1.888	12.161
-WD+M+I	5.868	2.498	1.460	9.825
-WD-M+I	8.670	3.094	1.851	13.615
-WD+M-I	7.487	3.430	1.913	12.830
-WD-M-I	9.700	4.008	2.129	15.837

Table-3. Chemical composition (g kg-1 DM), lignification and in vitro enzyme digestibility (%) of aboveground biomass of soybean, 2011-2012

Variants	CP	CF	NDF	ADF	ADL	C	Lighnif	IVDMD
+WD+M+I	153.0	282.9	437.8	421.6	63.5	358.01	14.50	63.76
+WD-M+I	158.5	274.0	435.8	325.8	67.3	258.5	15.40	67.32
+WD+M-I	137.8	256.0	420.2	340.3	89.3	251.0	21.20	65.38
+WD-M-I	163.0	264.1	336.6	333.9	57.7	276.2	17.10	64.26
-WD+M+I	170.6	247.6	575.9	338.1	67.7	270.4	11.80	66.50
-WD-M+I	182.8	240.0	382.3	314.5	67.8	246.7	17.70	65.10
-WD+M-I	171.2	266.6	410.8	352.2	71.6	280.6	17.40	67.09
-WD-M-I	167.2	256.9	441.1	343.3	70.4	272.9	16.00	67.18
CV	7.9	5.3	16.0	9.4	13.2	12.6	16.7	2.1

CP – Crude protein, CF – Crude fiber, NDF – Neutral-detergent fiber, ADF – Acid-detergent fiber, ADL – Acid-detergent lignin, C – Cellulose, Lignif – Degree of lignification (coefficient, ADL/NDFx100), IVDMD – In vitro dry matter digestibility

References

[1] Shao, H. B., Chu, L. Y., Jaleel, C. A., Manivannan, P., Panneerselvam, P., and Shao, M. A., 2009. "Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe." *Crit. Rev. Biotech.*, vol. 29, pp. 131-151.

[2] Cia, M. C., Guimarães, A. C. R., Medici, L. O., Chabregas, S. M., and Azevedo, R. A., 2012. "Antioxidant responses to water deficit by drought-tolerant and -sensitive sugarcane varieties." *Ann. Appl. Biol.*, vol. 161, pp. 313-324.

[3] Wang, W. X., Vinocur, B., and Altman, A., 2003. "Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance." *Planta*, vol. 218, pp. 1-14.

[4] Bray, E. A., 1997. "Plant responses to water deficit." *Trends Plant Sci.*, vol. 2, pp. 48-54.

[5] Subramanian, S. and Smith, D. L., 2013. "A proteomics approach to study soybean and its symbiont bradyrhizobium japonicum – a review." In *A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships (J. E. Board, Ed.), Quebec, Canada.* pp. 4-30.

[6] Racz, J., 2003. "Skúsenosti z pestovania sóje v poľno-klimatických podmienkach slovenska." *Agrochémia*, vol. 7, pp. 16-18.

[7] Sionit, N. and Kramer, P. J., 1977. "Effect of water stress during different stages of growth of soybeans." *Agron. J.*, vol. 69, pp. 274-278.

[8] Mederski, H. J., Jeffers, D. L., and Peterrs, D. B., 1973. *Water and water relations, in Soybeans: Improvement, production, and uses*. Madison, USA.

[9] Haile, F. J. and Higley, L. G., 2003. "Changes in soybean gas-exchange after moisture stress and spider mite injury." *Environ. Entomol.*, vol. 32, pp. 430-440.

[10] DeAngelis, J. D., Berry, R. E., and Krantz, G. W., 1983a. "Evidence for spider mite (Acari: Tetranychidae) injury induced leaf water deficits and osmotic adjustment in peppermint." *Environ. Entomol.*, vol. 12, pp. 336-339.

[11] DeAngelis, J. D., Berry, R. E., and Krantz, G. W., 1983b. "Photosynthesis, leaf conductance, and leaf chlorophyll content in spider mite-injured peppermint leaves." *Environ. Entomol.*, vol. 12, pp. 345-348.

[12] DeAngelis, J. D., Mafin, A. B., Berry, R. E., and Krantz, G. W., 1983c. "Effects of spider mite (Acari: Tetranychidae) injury on essential oil metabolism in peppermint." *Environ. Entomol.*, vol. 12, pp. 522-527.

[13] Lisar, S. Y. S., Motafakkerazad, R., Hossain, M. M., and M., R. I. M., 2012. *Water stress in plants: causes, effects and responses*. Croatia: InTech.

[14] Suekane, R., Degrande, P. E., de Melo, E. P., Bertoncello, T. F., S., d. L. J. I., and Kodama, C., 2012. "Damage level of the two-spotted spider mite Tetranychus urticae Koch (Acari: Tetranychidae) in soybeans." *Rev. Ceres.*, vol. 59, pp. 77-81.

[15] Youngman, R. R. and Barnes, M. M., 1986. "Interaction of spider mites (Acari: Tetranychidae) and water stress on gas-exchange rates and water potential of almond leaves." *Environ. Entomol.*, vol. 15, pp. 594-600.

[16] Smitley, D. R. and Peterson, N. C., 1996. "Interactions of water stress, honeylocust spider mites (Acari: Tetranychidae), early leaf abscission, and growth of Gleditsia triacanthos." *J. Econ. Entomol.*, vol. 89, pp. 1577-1581.

[17] Sadras, V. O., Wilson, L. J., and Lally, D. A., 1998a. "Dryland cotton tolerated mites better than irrigated cotton." In *IX Australian Cotton Conference. 12 -14 August 1998, Broadbeach, Australia.* pp. 423-426.

[18] Sadras, V. O., Wilson, L. J., and Lally, D. A., 1998b. "Water deficit enhanced cotton resistance to spider mite herbivory." *Ann. Bot.*, vol. 81, pp. 273-286.

[19] Küchenmeister, K., Küchenmeister, F., Kayser, M., Wrage-Mönnig, N., and Isselstein, J., 2013. "Influence of drought stress on nutritive value of perennial forage legumes." *Int. J. Plant Prod.*, vol. 7, pp. 693-710.

[20] Kaur, H., Sharma, P., and Sirhindi, G., 2013. "Sugar accumulation and its regulation by jasmonic acid in Brassica napus L. under salt stress." *J. Stress Physiol. Biochem.*, vol. 9, pp. 53-64.

[21] Abdelgawad, Z. A., Khalafaallah, A. A., and Abdallah, M. M., 2014. "Impact of methyl jasmonate on antioxidant activity and some biochemical aspects of maize plant grown under water stress condition." *Agric. Sci.*, vol. 5, pp. 1077-1088.

[22] Mohamed, H. I. and Latif, H. H., 2017. "Improvement of drought tolerance of soybean plants by using methyl jasmonate." *Physiol. Mol. Biol. Plants.*, vol. 23, pp. 545-556.

[23] Thielert, W., 2006. "A unique product: The story of the imidacloprid stress shield." *Pflanzenschutz-Nachr Bayer*, vol. 59, pp. 73-86.

[24] Febr, W. P. and Cavinesi, C. E., 1977. "Stages of soybean development." In *Ames, IA: Agriculture and Home Economics Experiment, Station and Cooperative Extension Service, Iowa State University*.

[25] Fischer, R. and Maurer, R., 1978. "Drought resistance in spring wheat cultivars. I. Grain yield responses." *Aust. J. Agric. Res.*, vol. 29, pp. 897-912.

[26] Barrs, H. D. and Weatherley, P. E., 1962. "A re-examination of the relative turgidity technique for estimating water deficit in leaves." *Aust. J. Biol. Sci.*, vol. 15, pp. 413-428.

[27] Ermakov, A., Arasimovich, V., Yarosh, N., Peruanski, Y., Lukovnikova, G., and Ikonomova, M., 1987. *Methods of biochemical surveys of plants*. Leningrad, Russia: Agropromizdat.

[28] Zelenskiy, M. and Mogileva, G., 1980. "Comparative evaluation of plant photosynthetic based on a photochemical activity of chloroplasts, Methodical instructions."

[29] Sinyakova, L. A. and Ivanova, A. I., 1981. *Methodological guidelines for determining the indicators of photosynthetic and root activity of plants*. Moscow.

[30] AOAC, 2010. *Official methods of analysis association of analytical Chemists*. Maryland, USA AOAC International.

[31] Todorov, N., Atanassov, A., Ilchev, A., Gantchev, G., Mihailova, G., Girginov, D., Penkov, D., Shindarska, Z., Naydenova, Y., *et al.*, 2010. *Practicum in animal nutrition*. Bulgaria: Sofia.

[32] Blum, A., 1997. *Crop responses to drought and the interpretation of adaptation, in drought tolerance in higher plants*. Dordrecht: Genetical, Physiological and Molecular Biological Analysis (E. Belhassen, Ed.). pp. 57-70.

[33] Quarrie, S. A. and Jones, H. G., 1977. "Effects of abscisic acid and water stress on development and morphology of whe at." *J. Exp. Bot.*, vol. 28, pp. 192-203.

[34] Bañon, S., Fernandez, J. A., Franco, J. A., Torrecillas, A., Alarcón, J. J., and Sánchez-Blanco, M. J., 2004. "Effects of water stress and night temperature preconditioning on water relations and morphological and anatomical changes of Lotus creticus plants." *Sci. Hortic.*, vol. 101, pp. 333-342.

[35] Stancheva, Y., 2000. *Ecological bases of agriculture*. Sofia: Pensoft.

[36] Grammatikopoulos, G. and Manetas, Y., 1994. "Direct absorption of water by hairy leaves of Phlomis fruticosa and its contribution to drought avoidance." *Can. J. Bot.*, vol. 72, pp. 1805-1811.

[37] Roy, B. A., Stanton, M. L., and Eppley, S. M., 1999. "Effects of environmental stress on leaf hair density and consequences for selection." *J. Evol. Biol.*, vol. 12, pp. 1089-1103.

[38] Hameed, M., Mansoor, U., Ashraf, M., and Rao, A., 2002. "Variation in leaf anatomy in wheat germplasm from varying drought-hit habitats." *Int. J. Agri. Biol.*, vol. 4, pp. 1-16.

[39] Gutiérrez-Boem, F. H. and Thomas, G. W., 2001. "Leaf area development in soybean as affected by phosphorus nutrition and water deficit." *J. Plant Nutr.,* vol. 24, pp. 1711-1729.

[40] Santos, R. F. and Carlesso, R., 1998. "Déficit hídrico e os processos morfológicos e fisiológicos das plantas." *R. Bras. Eng. Agríc. Ambiental.,* vol. 2, pp. 287-294.

[41] Catuchi, T. A., Vítolo, H. F., Bertolli, S. S., and Souza, G. M., 2011. "Tolerance to water deficiency between two soybean cultivars: transgenic versus conventional." *Cienc. Rural.,* vol. 31, pp. 373-378.

[42] Ohashi, Y., Saneoka, H., Matsumoto, K., Ogata, S., Premachandra, G. S., and Fujita, K., 1999. "Comparison of water stress effects on growth, leaf water status, and nitrogen fixation activity in tropical pasture legumes siratro and desmodium with soybean." *Soil Sci. Plant Nutr.,* vol. 45, pp. 795-802.

[43] Khan, M. S. A., Abdullah-Al-Mamun, M., Mahmud, A. A., Bazzaz, M. M., Hossain, A., Sultan Alam, M., Shamimuzzaman, M., and Abdul, M. K., 2014. "Effects of salt and water stress on leaf production, sodium and potassium ion accumulation in soybean." *J. Plant Sci.,* vol. 2, pp. 209-214.

[44] Mattson, W. J. and Haack, R. A., 1987. *The role of drought stress in provoking outbreaks of phytophagous insects*. Orlando: Academic Press.

[45] English-Loeb, G. M., 1989. "Nonlinear responses of spider mites to drought-stressed host plants." *Ecol. Entomol.,* vol. 14, pp. 45-55.

[46] Oi, D. H., Sanderson, J. P., Youngman, R. R., and Barnes, M. M., 1989. "Developmental times of the Pacific spider mite (Acari: Tetranychidae) on water-stressed almond trees." *Environ. Entomol.,* vol. 18, pp. 208-212.

[47] Huberty, A. F. and Denno, R. F., 2004. "Plant water stress and its consequences for herbivorous insects." *Ecology,* vol. 85, pp. 1383-1398.

[48] Jaleel, C. A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H. J., Somasundaram, R., and Panneerselvam, R., 2009. "Drought stress in plants: a review on morphological characteristics and pigments composition." *Int. J. Agric. Biol.,* vol. 11, pp. 100-105.

[49] Larcher, W., 2000. *Ecofisiologia Vegetal. Traduzido por PRADO, C. H. B. A. RiMa Artes e textos*. São Carlos.

[50] Paleg, L. G., Stewart, G. R., and Bradbeer, J. W., 1984. "Proline and glycine betaine influence protein solvation." *Plant Physiol.,* vol. 75, pp. 974-978.

[51] Zlatev, Z., 2005. "Effects of water stress on leaf water relations of young bean plants." *J. Cent. Eur.Agr.,* vol. 6, pp. 5-14.

[52] Jie, Y., Huang, P., and Li, Z., 2001. "Relationship between physiological-biochemical changes under drought stress under drought stress and drought resistance in Ramie." *Chinese Agricultural Sciences,* pp. 83-88.

[53] DeAngelis, J. D., Larson, K. C., Berry, R. E., and Krantz, G. W., 1982. "Effects of spider mite injury on transpiration and leaf water status in peppermint." *Environ. Entomol.,* vol. 11, pp. 975-978.

[54] Mefti, M., Abdelguerfi, A., and Chebouti, A., 2001. "Etude de la tolérance á la sécheresse chez quelques populations de Medicago truncatula (L.) Gaertn." *Options Méditerranéennes,* vol. 45, pp. 173-176.

[55] Gazanchian, A., Hajheidari, M., Sima, N. K., and Salekdeh, G. H., 2007. "Proteome response of Elymus elongatum to severe water stress and recovery." *J. Exp. Bot.,* vol. 58, pp. 291-300.

[56] Pinheiro, C., Chaves, M. M., and Ricardo, C. P., 2001. "Alterations in carbon and nitrogen metabolism induced by water deficit in the stems and leaves of Lupina albus L." *J. Exp. Bot.,* vol. 52, pp. 1063-1070.

[57] Al-Tabbal, J. A., Ayad, J. Y., and Kafawin, O. M., 2005. "Effect of water deficit and plant growth regulators on leaf chlorophyll, proline and total soluble sugar content of two durum wheat cultivars (Triticum turgidum L. var. durum)." *Agr. Sci.,* vol. 32, pp. 195-204.

[58] Nakayama, N., Saneoka, H., Moghaieb, R. E. A., Premachandra, G. S., and Fujita, K., 2007. "Response of growth, photosynthetic gas exchange, translocation of 13C-labelled photosynthate and N accumulation in two soybean (Glycine max L. Merrill) cultivars to drought stress." *Int. J. Agric. Biol.,* vol. 9, pp. 669-674.

[59] De Souza, G. M., Catuchi, T. A., Bertolli, S. C., and Soratto, R. P., 2013. "Soybean under water deficit: physiological and yield responses." In *In: A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships (J. E. Board, Ed.), Brazil.* pp. 273-298.

[60] Chaves, M. M., Flexas, J., and Pinheiro, C., 2009. "Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell." *Ann. Bot.,* vol. 103, pp. 551-560.

[61] Hildebrand, D. F., Rodriguez, J. G., Brown, G. C., and Volden, C. S., 1986. "Twospotted spider mite (Acari: Tetranychidae) infestations on soybeans: Effect on composition and growth of susceptible and resistant cultivars." *J. Econ. Entomol.,* vol. 79, pp. 915-921.

[62] Wheeler, A. G., 2001. *Biology of the plant bugs (Hemiptera: Miridae): pests, predators, opportunists*. Ithaca, New York: Cornell University Press.

[63] Sailaja, D., Lakshmi, V., Praveen, M. V., Rao, D. R., Jagadeesh, S., Kumar, T. R., Adithyayadav, M., and Chary, T. P., 2012. "Estimation of levels of carbohydrates in Mealey bug (Maconellicoccus hirsutus) infected stem of Hibiskus Rosa sinesis." *Int. J. Appl. Biol. Pharm. Tech.,* vol. 3, pp. 410-413.

[64] Tomczyk, A., 2013. "Changes in the content of soluble sugars in leaves of cucumber plant infested with Tetranychus urticae Koch and treated with plant growth promoting rhizobacteria (PGRP)." In *In: Acarid Phylogeny and Evolution: Adaptation in Mites and Ticks (F. Bernini, R. Nannelli, G. Nuzzaci, E. Lillo, Eds.).* pp. 430-451.

[65] Brevedan, R. E. and Egli, D. B., 2003. "Short periods of water stress during seed filling, leaf senescence and yield of soybean." *Crop Science Society of America,* vol. 43, pp. 2083-2088.

[66] Inamullah, I. A., 2005. "Adaptive responses of soybean and cotton to water stress. II. Changes in CO2 assimilation rate, chlorophyll fluorescence and photochemical reflectance index in relation to leaf temperature." *Plant Prod. Sci.,* vol. 8, pp. 131-138.

[67] Krivosudská, E. and Filová, A., 2013. "Evaluation of selected soybean genotypes (Glycine max L.) by physiological responses during water deficit." *J. Cent. Eur. Agr.,* vol. 14, pp. 213-228.

[68] Reynolds, M. P., Kazi, A. M., and Sawkins, M., 2005. "Prospects for utilizing plant adaptive mechanisms to improve wheat and other crops in drought and salinity prone environments." *Ann. Appl. Biol.,* vol. 146, pp. 239-259.

[69] Allakhverdiev, S. I., Sakamoto, A., Nishiyama, Y., and Murata, N., 2000. "In activation of photosystems I and II in response to osmotic stress in Synechococcus. Contribution of water channels." *Plant Physiol,* vol. 122, pp. 1201-1208.

[70] ShiraniRad, A. H. and Zandi, P., 2012. "The effect of drought stress on qualitative and quantitative traits of spring rapeseed (Brassica napus L.) cultivars." *Žemdirbyst,* vol. 99, pp. 47-54.

[71] Tambussi, E. A., Bartoli, C. G., Bettran, J., Guiamet, J. J., and Araus, J. C., 2000. "Oxidative damage to thylakoids proteins in water stressed leaves of wheat (Triticum aestivum L.)." *Physiol. Plant.,* vol. 108, pp. 398-404.

[72] Bueno, A. F., Bueno, R. C. O. F., Nabity, P. D., Higley, L. G., and Fernandes, O. A., 2009. "Photosynthetic response of soybean to twospotted spider mite (Acari: Tetranychydae) injury." *Braz. Arch. Biol. Technol.,* vol. 52, pp. 825-834.

[73] Vassilev, A. and Koleva, M., 2010. "Photosynthesis response of plants from three cotton cultivars to soil drought." *Scientific works-AU Plovdiv,* vol. 1, pp. 195-202.

[74] Han, S. H., Kim, C. H., Lee, J. H., Kim, I. S., and Kim, Y., 2010. "Induced drought tolerance by the insecticide imidacloprid in plant." *Korean J. Environ. Agric.,* vol. 29, pp. 159-164.

[75] Emam, M. M., Khattab, H. E., Helal, N. M., and Deraz, A. E., 2014. "Effect of selenium and silicon on yield quality of rice plant grown under drought stress." *Aust. J. Crop Sci.,* vol. 8, pp. 596-605.

[76] Moorby, J. M., Evans, R. T., Scollan, N. D., Mac Rae, J. C., and Theodorou, M. K., 2006. "Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.). Evaluation in dairy cows in early lactation." *Grass Forage Sci.,* vol. 61, pp. 52-59.

[77] Peterson, P. R., Sheaffer, C. C., and Hall, M. H., 1992. "Drought effects on perennial forage legume yield and quality." *Agron. J.,* vol. 84, pp. 774-779.

[78] Buxton, D. R. and Mertens, D. R., 1996. *Quality-related characteristics of forages. In forages, (R.F. Barnes, D. A. Miller and C. J. Nelson, Eds.)* vol. 2. Iova, pp. 83-96.

[79] Dhooria, M. S. and Bhullar, M. B., 2007. "Integrated pest management of Phytophagus mites." In *In: Entomology: Novel Approaches (P. C. Jain and M. C. Bhargava, Eds.), New Delhi.* pp. 461-476.

[80] Rethwisch, M. D., Bolin, K., Grudovich, J. L., Wellman, J., Van Dyke, C., Vingochea, J., Barron, M., and Reay, M., 2004. "Effects of two-spotted spider mites and miticides on alfalfa hay produced for a late may cutting university of arizona college of agriculture forage and grain report."

[81] Stiling, P., 2009. *Greenhouse gases, global warming and insects*. California: Encyclopedia of Insects (V.H. Resh and R.T. Cardé, Eds.). pp. 428-431.

[82] Taiz, L. and Zeiger, E., 2006. *Plant physiology*. Massachusetts, USA: Sinauer Associates.

[83] Georgieva, N., Nikolova, I., and Ilieva, A., 2012. "Influence of treatment with Confidor 70 WG on productivity and chemical composition of winter vetch (Vicia villosa Roth.) in different sowing time." *Plant Sci.,* vol. 49, pp. 48-54.